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BRIEF COMMUNICATION

Effects of enhanced UV-B radiation and tropospheric ozone on physiological and biochemical characteristics of field grown wheat

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Abstract

Experiments were conducted under field conditions to assess the growth, physiological and biochemical responses of wheat plants (*Triticum aestivum* L.) to supplemental UV-B radiation (7.1 kJ m^{-2}) and enhanced ozone (0.07 μmol mol^{-1}) separately and in combination. Enhanced UV-B radiation and O_3 reduced biomass, yield, photosynthetic rate, chlorophyll, carotenoid and ascorbic acid contents and catalase activity, whereas increased total phenol content and peroxidase activity. Contents of flavonoids increased due to UV-B treatment. The interactive effects were, however, less than additive.

Additional key words: carotenoids, catalase, chlorophyll, peroxidase, phenol content, photosynthetic rate, stomatal conductance, *Triticum aestivum*.

During the last two decades, significant reductions in the concentrations of stratospheric O_3 have been reported. This reduction causes an increment in ultraviolet-B radiation approaching the Earth. An increase in concentration of UV-B flux densities at Earth's surface can result in net increase in local tropospheric O_3 concentrations in urban environment supplied with oxides of nitrogen, methane and carbon monoxide (Caldwell *et al.* 1998). UV-B radiation and increased tropospheric O_3 concentrations may occur together and cause reductions in physiological and biochemical characteristics of economically important plants (Miller *et al.* 1994). UV-B radiation is known to affect the leaf area, stem growth, protein content (Caldwell *et al.* 1998) and net photosynthesis (Ambasht and Agrawal 1998). The effects of UV-B and O_3 on plant growth and productivity have been reported separately for a large number of species, but only few experiments have focused on their interaction (Ormrod *et al.* 1995, Zeuthan *et al.* 1997). The studies on combination of O_3 and UV-B were mostly conducted in temperate mid latitude countries, whereas tropospheric O_3 concentrations are higher in tropical countries.

The present study was aimed to assess the impact of elevated levels of O_3 and UV-B individually and in combination on physiological and biochemical characteristics, biomass and yield of wheat plants (*Triticum aestivum* L. cv. Malviya 234). The present work is the first report on interactive effects of O_3 and UV-B on wheat plants from tropical region conducted under field conditions.

The field experiments were conducted from November through mid of April at Botanical garden of Banaras Hindu University, Varanasi (25° 18 'N, 83° 1 'E about 76 m above mean sea level) situated in the eastern Gangetic plains of India. Soil of the study site was sandy loam in texture (sand 45 %, silt 28 % and clay 27 %) and neutral pH (7.2 to 7.4). During the experiment, temperature ranged from 10 to 36 °C, relative humidity from 70 to 98 % and rainfall was 172 mm. Photosynthetically active radiation (PAR) averaged 990 μmol m^{-2} s^{-1} at midday. Wheat [*Triticum aestivum* (L.) cv. M 234] seeds were sown 20 cm apart in rows in 12 plots of 1.5 × 1.5 m each. Recommended doses of nitrogen, phosphorus and potassium as urea, super

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Abbreviations: AA - ascorbic acid; AC - anthocyanins; Car - carotenoids; CAT - catalase; Chl - chlorophylls; FL - flavonoids; g_s - stomatal conductance; P_N - net photosynthetic rate; POX - peroxidase.

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phosphate and muriate of potash (120:80:40 kg ha^{-1}) were amended to all plots. Twelve plots were randomly divided into four treatments, *i.e.* control (C), UV-B treated (UV-B), O_3 treated (O_3) and combination of UV-B and O_3 (UV-B+O_3). Elevated UV-B was artificially provided by *Q-Panel UV-B 313* 40 W fluorescent lamps (*Q Panel Inc.*, Cleveland, OH, USA). Four lamps per bank fitted 30 cm apart on a wooden frame were suspended 0.45 m above and perpendicular to the planted rows (Ambasht and Agrawal 1997, 1998). Plants were artificially irradiated for 5 h per day in the middle of the photoperiod from germination (0 d) to maturity (110 d). The 0.45 m distance between the top of plant canopy and UV-B lamps was kept constant throughout the plant life. Each lamp was covered with 0.13 mm thick cellulose diacetate film (*Cardillac Plastics*, Baltimore, U.S.A) which absorbed radiation emitted by lamps below 290 nm. For control, lamps were covered with 0.13 mm thick polyester film (*Cardillac Plastics*) which absorbed radiation emitted by lamps below 320 nm. The UV-B irradiance at the top of the canopy under the lamps was measured by an Ultraviolet intensity meter (*UVP Inc.*, San Gabriel, CA, USA). The readings were converted to $UV\text{-}B_{BE}$ values by comparing UV meter readings with a *Spectro Power Meter* (*Scientech Inc.*, Boulder, CO, USA). Plants beneath cellulose diacetate film received 7.1 kJ m^{-2} UV-B radiation. This enhanced level of UV-B over the ambient was similar to those, which mimicked 15 % reduction in stratospheric ozone at Varanasi (25°N) during clear sky condition on the summer solstice normalized at 300 nm.

Ozone was generated by an ozonator (*Standard Appliances*, *Model SA-112-Lp-230C*, Varanasi, India). Plants were exposed to 0.07 µmol mol^{-1} O_3 for 4 h per day up to grain maturity (110 d). Exposure was done in a 1.5 m^2 open top chamber covered with 0.25 mm thick transparent polythene supported on an iron frame. Each chamber was fed with an additional flow of air (56 $dm^{-3}s^{-1}$) through a heavy-duty blower, which led to one total air exchange min^{-1}. The delivery end of the ozonator was connected to the distribution system so that the diluent air coming from the blower circulates the O_3 gas into the chamber. Ozone was monitored by sucking known amount of air from chamber and absorbing it into buffered potassium iodide solution (0.1 M), and the absorbance was taken at 340 nm using UV-Vis spectrophotometer (Model 119 Systronics, New Delhi, India). Ozone concentration was calculated using the method of Byers and Saltzman (1958).

Three plants (60-d-old) were sampled randomly from each replicate plots for all the analyses. Chlorophyll and carotenoid were extracted from the leaf discs with 80 % acetone and quantified as described by Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Estimation of anthocyanin (AC) and flavonoid (FL) contents were done by using the methods of Beggs and Wellmann (1985) and Flint *et al.* (1985), respectively. Total phenol and ascorbic acid (AA) contents were quantified using the methods of Bray and Thorpe (1954) and Keller and Schwager (1977), respectively. The catalase (CAT, EC 1.11.1.6) and peroxidase (POX, EC 1.11.1.7) were extracted by homogenizing leaf samples in 0.1 M cold phosphate buffer (pH 7) containing 5 mM cysteine at 4 °C. The activities were quantified as described by Kar and Mishra (1976) and Britton and Mehley (1955), respectively. Net photosynthetic rate (P_N) rate and stomatal conductance (g_s) were measured with portable photosynthesis system (*LI 6200*, *LI-COR*, Lincoln, NE, USA) on the intact, fully expanded third leaf below the top under ambient conditions at a PAR between 1000 - 1200 µmol m^{-2} s^{-1} and mean ambient CO_2 concentration of 350 µmol mol^{-1}. For biomass determination plants were harvested, carefully washed and then oven dried at 80 °C to constant mass. Seed yield (g m^{-2}) was calculated at the time of harvest (110 d). Quantitative changes in different parameters were analyzed through Analysis of Variance test. Duncan's Multiple Range test was applied for mean separation for significant differences among treatments.

P_N declined significantly in O_3 (13.4 %) and UV-B+O_3 treated plants (22.06 %) while g_s only in UV-B+O_3 treated plants (Table 1). The combined effect of O_3 and UV-B was less than additive for both photosynthetic rate and stomatal conductance. O_3 induced stomatal closure and consequent decrease in P_N was described by Darrall (1989). Miller *et al.* (1994), however, did not find significant changes in these parameters due to individual and combined treatments of UV-B and O_3. Biomass accumulation and yield were lower in all the treatments as compared to the control, but the reductions were significant only at combined treatment (Table 1). Significant reductions in biomass and yield of *Oryza sativa* plants exposed to UV-B radiation at 15 % (Ziska and Teramura 1992) and 20 % (Ambasht and Agrawal 1997) ozone depletion were reported. Ormrod *et al.* (1995) also found reductions in dry mass of *Arabidopsis* plants after UV-B and O_3 exposure separately and in combination. Yue *et al.* (1998) have reported 45.7 % reduction in biomass of UV-B treated (5.31 kJ m^{-2}) wheat plants. However, Teramura *et al.* (1990) have found no reductions in growth and yield of wheat and rice grown under greenhouse and exposed to UV-B radiation at 10 % ozone depletion.

Chl content declined significantly in response to UV-B and in UV-B+O_3 treatments, the reduction was maximum in combined treatment (Table 1). Chl is destroyed by UV-B radiation (He *et al.* 1994) as well as by O_3 exposure (Teramura *et al.* 1990, Kangasjarvi *et al.* 1994). Strid *et al.* (1990) found reduction in Car content of UV-B treated pea plants. Car protect photosynthetic membranes against internally and externally generated photoxidative products. Reduction of their content may lead to decline in photoprotection and in consequence to photoinhibition.

Table 1. Effects of enhanced UV-B radiation and ozone separately and in combination on net photosynthetic rate, stomatal conductance, biomass, yield, contents of chlorophyll, carotenoids, anthocyanin, flavonoid and ascorbic acid and activities of catalase and peroxidase of *T. aestivum* plants. Means ± SE, $n = 9$, values marked with the same letter are not significantly different from each other at $P < 0.05$.

Parameters	Control	UV-B	O_3	UV-B+O_3
Photosynthesis [μmol(CO_2) m^{-2} s^{-1}]	28.23 ± 1.02[a]	25.62 ± 1.34[a]	24.45 ± 0.79[b]	22.00 ± 1.67[b]
Stomatal conductance [cm s^{-1}]	2.08 ± 0.04[a]	1.92 ± 0.02[a]	1.78 ± 0.05[a]	1.19 ± 0.07[b]
Biomass [g $plant^{-1}$]	24.34 ± 1.25[a]	22.84 ± 1.56[a]	21.45 ± 0.98[a]	19.32 ± 1.34[b]
Yield [g m^{-2}]	472.52 ± 7.12[a]	452.20 ± 6.56[a]	431.00 ± 6.93[a]	428.00 ± 7.78[b]
Chlorophyll content [mg g^{-1}(d.m)]	2.58 ± 0.15[a]	1.95 ± 0.02[b]	2.12 ± 0.12[a]	2.06 ± 0.16[b]
Carotenoid content [mg g^{-1}(d.m)]	2.85 ± 0.32[a]	2.34 ± 0.04[b]	2.31 ± 0.08[b]	1.71 ± 0.04[b]
Anthocyanin content [mg g^{-1}(f.m)]	0.46 ± 0.01[a]	0.52 ± 0.09[a]	0.48 ± 0.02[a]	0.57 ± 0.08[a]
Flavonoid content [A_{300} mg^{-1}(f.m)]	1.17 ± 0.01[a]	1.56 ± 0.04[b]	1.24 ± 0.04[a]	1.58 ± 0.08[b]
Phenol content [mg g^{-1}(f.m)]	7.70 ± 1.04[a]	9.50 ± 1.05[b]	8.30 ± 1.14[a]	9.80 ± 0.34[b]
Ascorbic acid content [mg g^{-1}(f.m)]	2.40 ± 0.084[a]	0.80 ± 0.07[b]	1.80 ± 0.24[b]	1.04 ± 0.02[b]
CAT activity [μmol(H_2O_2) g^{-1}(f.m) s^{-1}]	6.24 ± 1.01[a]	4.75 ± 0.056[b]	5.48 ± 0.89[b]	4.28 ± 0.58[b]
POX activity [μmol(purpurogallin) g^{-1}(f.m) s^{-1}]	24.50 ± 2.31[a]	68.40 ± 2.47[b]	54.20 ± 3.01[b]	73.70 ± 4.23[b]

AC, a weak protective pigment against UV-B did not increase significantly due to treatments (Table 1). FL are known to provide protective function as an optical screen to UV-B radiation (Tevini and Teramura 1989). Teramura *et al.* (1990) have found 42 % increase in UV-B absorbing compounds at enhanced UV-B exposure. In the present study FL content increased by 33.3 % in UV-B and 35 % in UV-B+O_3 treated plants as compared to the control (Table 1). Phenols are precursors of FL. In the present study total phenol content increased maximally in UV-B+O_3 treated plants (Table 1).

CAT activity decreased with increase in POX activity after UV-B and O_3 exposure both separately and in combination (Table 1). O_3 induced increase in POX activity has often been reported (Kangasjarvi *et al.* 1994, Rao *et al.* 1996). Willekens *et al.* (1994) suggested that the effects of O_3 and UV-B on the antioxidant genes are very similar. Ambasht and Agrawal (1997) found increase in POX activity in leaves of field grown rice treated with UV-B radiation simulating 20 % O_3 depletion. AA content declined significantly in all sets of treatments as compared to the control suggesting the increase of oxidative stress upon UV-B and O_3 treatments separately and in combination (Table 1). AA is an important antioxidant, maintaining plant cell stability during stress condition by scavenging cytotoxic free radicals (Halliwell and Gutteridge 1989).

In the present study enhanced UV-B simulating 15 % O_3 depletions did not significantly reduce P_N, g_s, biomass and yield of wheat plants. The wheat resistance to UV-B was probably due to increased leaf flavonoid contents. O_3 caused significant reduction in P_N. Combined treatment of both these stresses showed maximum unfavorable effects on the measured parameters. Observed effects of UV-B+O_3 combination, however, were less than additive. This study further suggests that UV-B, O_3 and combined treatments induced oxidative stress in plants.

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